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Defensive Secretions of Amazonian Opilionids from Republic of Ecuador †

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Abstract: About 120 species of opilionids have been described in Ecuador (7000 worldwide), The Ecuadorian Amazon is the region where the greatest number of species is concentrated. Opilionids inhabit humid and shaded habitats and are generally nocturnal. these species possess glands secreting defensive secretions containing benzoquinonoids (benzoquinones and poly-substituted phenols). In this study we report the defensive compositions of neotropical opilionids inhabiting the Arajuno region, Ecuador, at the Juri Juri Kawsay Scientific Station of the Central University of Ecuador. The GC examination of the extract obtained from the defensive secretion of these Gonyleptidae (Order Laniatores, Opilinida) revealed the presence of five volatile components, three benzoquinones and two hydroquinones, being the major component 2,3-dimethyl-1,4-benzoquinone. A wide variety of benzoquinonoids have been characterized from plant and microbial sources, as well as from marine organisms. Among terrestrial invertebrates, benzoquinonoids are widely biosynthesized by arthropods including, besides millipedes, insects and opilionids. There seems little doubt that in theses invertebrates the benzoquinonoids secretions serve to repel predators and pathogenic microorganisms.

Keywords: opilionids; Ecuadorian Amazonia; defensive secretions; benzoquinonoids

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1. Introduction

According to Ecuadorian Entomological Society, in Ecuador there are approximately 185 described species of Opiliones (harvestmen) and 15 families. The families with the highest number of species are Cranaidae, Cosmetidae and Gonyleptidae with 75, 55 and 13 species respectively [1]. The Amazon region of Ecuador concentrates the largest number of known described species, 120, where the great majority of them occur in the provinces of Pastaza, Napo and Sucumbíos.

All three sub-orders of the Opiliones-the Cyphophthalmini, the Laniatores, and the Palpatores-have defensive prosomal exocrine glands. Chemical studies of opilionid defensive secretions have not been extensive, including taxa from Ecuadorian Amazonia. Laniatores, produce some alkylated 1,4-benzoquinones (2,3-dimethyl-1,4-benzoquinone and related substituted ones), and phenols (dimethyl substituted). Other components have been detected in defensive secretions of this invertebrates, such as: volatile branched chain alcohols, dienyl alcohols, bornyl esters, aromatic amines, ketones and aldehydes [2–4]. These repugnatorial secretions among their defensive significance against predators, constitute an eco-sustainable source of biologically active secondary metabolites, benzoquinonoid type, with potential broad-spectrum of microbiocidal action [5,6].

The chemical composition of defensive exocrine secretions from Opilionids of Ecuadorian Amazonia have not been previously described. In this context, de main goal of the present communication is to report the defensive compositions of neotropical opilionids inhabiting the Arajuno region, Pastaza Province, Ecuador, at the Juri Juri Kawsay Scientific Station of the Central University of Ecuador.

2. Materials and Methods

2.1. Location of Collected Individuals of Opilionids

Study area—The study area was the "Juri Juri Kawsay" Scientific Scientific Station "Juri Juri Kawsay" located in the Province of the Province of Pastaza, Ecuador, Canton Arajuno, Ethnoecological Community Pablo Lopez de Oglán Alto (CEPLOA) in the Alto (CEPLOA) in the upper basin of the Oglán River. The collection station was geo-referenced with the use of global positioning using a global positioning system (GPS) Garmin eTrex 30 and the coordinates and altitudes of the collection station were: 77°41′18.8″—01°19′ 25.0″ at 581 m above sea level.

2.2. Biological Material

For eliciting de repugnatorial secretion the procedure is quite simple, just by hand gently or by using a fine forceps. After disturbing the opilionid, appears a clear fluid forming some small droplets odorless. This liquid, after several minutes of being exposed to atmospheric conditions, becomes opalescent and yellowish with a very unpleasant odor. Defensive secretion (450 μL/ind.) was collected from five individuals (Gonyleptida, Laniatores, shown in Figure 1) in vivo and subsequently they were released. The ejected secretion was collected on Whatman-4 filter paper, stored at −10 °C, extracted from the filter paper with diethyl ether and concentrated with nitrogen flow to dryness (5,5 mg) and then was stored at −10 °C.

Figure 1. Individual of Gonileptidae-Laniatores Opilionid in the collection station of Juri-Juri-Kawsay, Pastaza, Ecuador. (Photo JETM).

The general chart flow of operations for studying defensive secretions isolated from Opilionids collected at Scientific Station Juri-Juri-Kawsay, Pastaza, Ecuador is depicted in Figure 2.

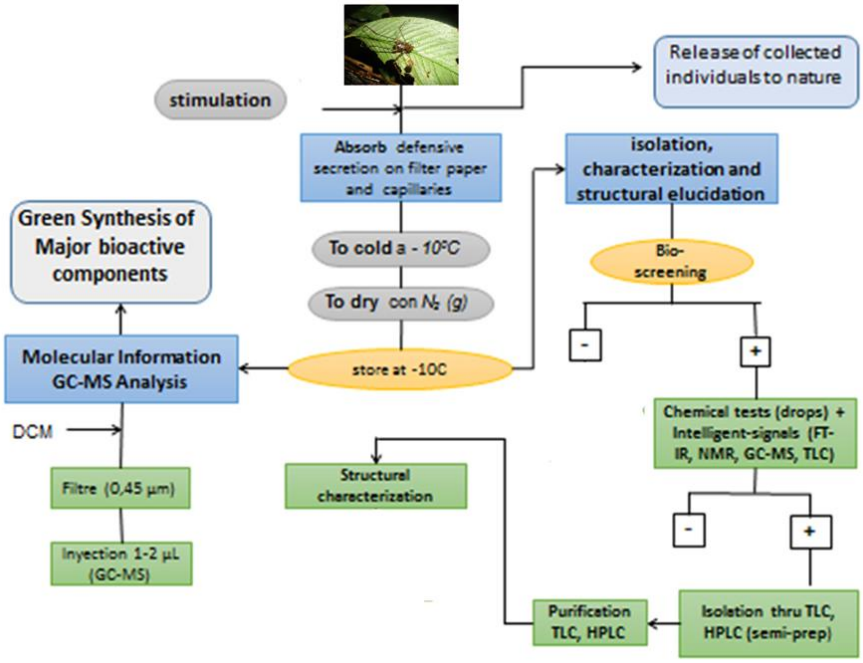

Figure 2. General protocol for studying defensive secretions isolated from Opilionids collected at Scientific Station Juri-Juri-Kawsay, Pastaza, Ecuador.

All reagents used were supplied by MERCK, Darmstadt, Germany, and were used without prior purification.

2.3. FTIR Spectroscopy

The infrared spectra were recorded on a PHILIPS ANALYTICAL FTIR PU-9600 spectrophotometer, Germany; the samples were prepared in potassium bromide (KBr) tablets at 25 °C. Alternatively, the spectra were recorded in a JASCO-Canvas 4600, Japan system in CsBr tablets at 25 °C.

2.4. Gas Chromatography Coupled to Mass Spectrometry (GC-MS)

For the determinations of the volatile poly-component mixtures of the repugnatorial secretions, a quadrupole GC-MS HP 6890 detection system (Palo Alto, CA, USA) was used. The separation technique, instrumental conditions and protocols were developed according to [7]. For molecular characterization of main components of the defensive opilionid ejections, m/e data from GC-MS analyses and comparison of their retention times and mass spectra, as well as related information in databases and molecular libraries, were used as described in [7].

3. Results and Discussion

The lack of compositional molecular data on the repugnatorial secretions of Amazonian opilionids from Ecuador is, therefore, the basis for this preliminary study. The preliminary taxonomic description of the collected individual reports that it belongs to Gonyleptidae-Laniatores. The analysis, by thin layer chromatography (SiO_2 plates doped with silver nitrate and the ability of the secretion to react strongly with KI starch paper suggested the presence of quinones), of the repugnatorial secretions, collected *in situ*, of the populations under study of Amazonian Gonyleptid opilionids from Scientific Station Juri Juri Kawsay, Ecuador, shown that all defensive secretions are poly-component mixtures of quinonoid metabolites (phenols and benzoquinones). The compositional-structural analysis, by GC-MS and FTIR, of the Gonyleptid opilionid defensive secretion revealed a very interesting molecular pattern.

In the registered FTIR spectrum, a broad band was observed at 3347 cm^{-1} corresponding to OH valence vibration and another one in the zone of in-plane bends at 12,384 cm^{-1} (σ CO), being both signals typical of phenols-hydroquinones. Characteristic bands of aromatics were also observed in the range 3050–3100 cm^{-1} (νCsp2-H) and 1450–1665 cm^{-1}. The presence of a significative and intense signals in the region of the CO valence vibrations (1645 y 1670 cm^{-1}, carbonyl) demonstrated of the presence of quinones as major metabolites in the defensive secretion.

The analysis of volatile compounds by GC/MS using a simple protocol: 1 mg of dry extract was dissolved in 1 mL of chloroform (1000 µg/mL), diluted with chloroform to 50 µg/mL and injected 2 µL at 280 °C, allowed the identification of the main benzoquinonoids (phenols and benzoquinones) present in the defensive secretion.

Taking into account the obtained results described, a molecular distribution pattern of quinonoid molecular systems (substituted phenols and 1,4-benzoquinones) for defensive secretion of Amazonian opilionids collected in the Scientific Station of Juri Juri Kawsay, Ecuador, was inferred and depicted in Figure 3.

Figure 3. Chemical composition of Gonyleptid Opilionid from Ecuadorian Amazonia, Scientific Station Juri Juri Kawsay, Arajuno, Pastaza, Ecuador. On the basis of CG/MS data analysis.

It should be noted that these major benzoquinonoid components are widely distributed among terrestrial invertebrates, including opilionids, coleoptera, and Diplopoda, revealing their potential chemical taxonomic significance [8–10].

4. Conclusions

The analysis (FTIR-GC/MS) of the defensive repugnatorial secretion of the Gonyleptid Opilionid, inhabiting in the Amazonian region of Arajuno, Pastaza Province, Republic of Ecuador, reveled the quinonoid composition of the secretion. Was identified 2,3-dimethyl-1,4-benzoquinon as a major component. This is the first report related to a preliminary analysis of chemical composition of repugnatorial secretion of Ecuadorian Gonyleptid Opilionid from Amazonian region. The results concerning biological action (repellent action against predator and epidemiological vectors and microbiocidal effects) and synthetic protocol in eco-sustainable conditions will be reported soon.

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